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The domain *Archaea* in human mucosal surfaces

F. Matarazzo[1,2], A. C. Ribeiro[1], M. Faveri[1,3], C. Taddei[4,5], M. B. Martinez[5] and M. P. A. Mayer[2]

1) *Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP,* 2) *Department of Dentistry, Centre of Health Science, State University of Maringá, Maringá, PR,* 3) *Dental Research Division, Department of Periodontology, University of Guarulhos, Guarulhos, SP,* 4) *School of Arts, Sciences and Humanities, University of São Paulo, São Paulo, SP and* 5) *Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil*

Abstract

Archaea present distinct features from bacteria and eukaryotes, and thus constitute one of the branches of the phylogenetic tree of life. Members of this domain colonize distinct niches in the human body, arranged in complex communities, especially in the intestines and the oral cavity. The diversity of archaea within these niches is limited to a few phylotypes, constituted in particular by methane-producing archaeal organisms. Although they are possibly symbionts, methanogens may play a role in the establishment of mucosal diseases by favouring the growth of certain bacterial groups.

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Corresponding author: M. P. A. Mayer, Department of Microbiology, Institute of Biomedical Sciences, Av. Lineu Prestes, 1374, São Paulo, SP 05508-900, Brazil
E-mail: mpamayer@icb.usp.br

Introduction

Members of the domain *Archaea* are prokaryotes that constitute one of the branches of the phylogenetic tree of life, the others being the domains *Bacteria* and *Eukarya*. Although the name *Archaea* was proposed by Woese in 1977 [1], the third domain of life was only accepted after molecular methods showed significant differences in ribosomal RNAs, ribosomal proteins and the composition of cell surface between members of the *Archaea* and *Bacteria* or *Eukarya* [2,3].

Numerous components of the archaeal informational processes are more similar to their eukaryotic than bacterial homologues [4]. For instance, archaeal genomes encode homologues of nearly all eukaryotic DNA replication proteins, but only one homologue of a bacterial DNA replication protein [2].

On the other hand, the lipids of archaea are unique to this domain, being formed by isoprenoid side chains ether-linked to the glycerol moiety, instead of glycerol ester lipids as found in bacteria [5,6]. Furthermore, the cell wall of archaea is fundamentally different from the bacterial cell wall. Studies on archaeal cell surface structures have revealed a lack of peptidoglycan [7], although a few archaea may possess cell envelope polymers formed by pseudomurein, similar to bacterial peptidoglycan, whereas *Methanosarcina* spp. have methanochondroitin, similar to the chondroitin produced by vertebrates. The cell surface of most archaeal organisms has a crystalline protein layer (S-layer), often composed of a single protein or glycoprotein, which is associated with the cytoplasmic membrane. By contrast, members of the *Thermoplasmatales*, which live under extreme conditions, do not possess a cell wall [6,8].

Because archaea lack peptidoglycan, their susceptibility to antibiotics is quite particular. Methanogenic archaea were found to be resistant to antibiotics used for for bacteria that target RNA synthesis and inhibit the synthesis or cross-linkage of the peptide subunit of murein, such as penicillin, cephalosporin, glycopeptides, and aminoglycosides. Despite their differences in membrane lipids, archaea are susceptible to antibiotics that inhibit the lipid cycle in bacteria, such as bacitracin, chloramphenicol, lasalocid, and monesin [9].

Surface appendages play an important role in the spatial organization of cells, from initial surface attachment to the development of mature community structures. Very little is known about the morphology and function of surface appendages of archaea, but members of this domain have been frequently detected in mixed communities in aggregates [10] and complex biofilms [11–14]. Thus, it is conceivable that these structures may have roles in locally increasing the concentrations of substrates around the cell [15], movement, and co-aggregation with other microorganisms or adhesion to abiotic and biotic surfaces [6,8]. Interestingly, genes encoding class III signal peptides, similar to those encoding bacterial type IV pilins, were found in the genomes of several archaea, indicating that as yet undescribed cell surface structures, such as archaeal pili, may be present in most members of this domain [16]. In fact, pili-like structures are involved in biofilm formation by the thermoacidophilic crenarchaeote *Sulfolobus acidocaldarius* [17], and in the aggregation of *Methanothermobacter thermautotrophicus* with bacteria, conferring more efficient H_2 transfer [18].

Archaea are frequently referred to as 'extremophiles', because of their presence in extreme environments with respect to temperature, osmotic pressure, salinity, and pH values [19,20]. This terminology has been shown to be inappropriate by further research, as members of this domain have been detected in a wide range of habitats, including the mucosal surfaces of mammals.

These organisms could be physiologically classified into: methanogens (strict anaerobes that produce methane); halophiles (strict aerobes living in highly salty environments); and thermoacidophiles (aerobes living in hot and acidic environments).

The domain *Archaea* is composed of four phyla: the *Euryarchaeota*, the *Crenarchaeota*, the *Korarchaeota*, and the *Nanoarchaeota*. The main archaea detected in humans and animals belong to the phylum *Euryarchaeota*, which includes the five known orders of methanogens (*Halobacteriales*, *Methanobacteriales*, *Methanosarcinales*, *Methanomicrobiales*, and *Methanococcales*) [21]; a sixth order has also been proposed [22]. Methanogenesis is exclusively related to archaeal organisms [2], and the methanogenic archaea are considered to be the only biological source of methane [23]. These organisms participate in the conversion of organic matter, under anaerobic conditions, by utilizing the metabolic products of bacteria (i.e. CO_2, H_2, acetate, and formate) and other methyl compounds available in the environment, converting them to methane [21,23].

Human microbiome studies have revealed that archaea colonize distinct niches in the human body, arranged in complex communities [24–27]. Archaea are mainly found in the gut [13,14,28–30] and the oral cavity [11,12,31–33]. Overall, archaeal diversity is limited to a few phylotypes in the human microbiota (i.e. vagina, intestinal tract, and oral cavity) [21,34]. *Methanobrevibacter smithii* is the most prevalent species in the gut, followed by *Methanosphaera stadtmanae* [35], whereas *Methanobrevibacter oralis* is the main species in the oral cavity [12,36]. However, recent molecular approaches have demonstrated that *Methanosarcina*, *Thermoplasma*, members of the *Crenarchaeota* and halophilic archaea are also found in the gastrointestinal tract [9]. The *Halobacteriaceae* family, consisting of aerobic halophiles that require a hypersaline environment, was detected at low density as compared with the methanoarchaeal population in the gut microbiota [37,38], and the presence of members of this family might be associated with a high-salt-containing diet.

Most archaea inhabiting human surfaces, such as *M. smithii* and *M. oralis*, obtain energy for growth only by the reduction of CO_2 to methane, and are referred to as obligate CO_2-reducing species [39]. Others, like the genus *Methanosphaera*, reduce the methyl group of methanol to methane [34]. Furthermore, members of the genus *Methanosarcina* obtain energy for growth by producing methane from dismutation of the methyl group of methanol and methylamines, but also by reducing CO_2, with H_2 or CO as the electron donor, and by converting acetate to methane and CO_2 [39]. In the mixed communities of the intestinal tract, the resulting methane is used as a source of carbon and energy by some aerobic alpha-proteobacteria and delta-proteobacteria [40,41], and may be exhaled as methane breath [39].

Archaeal metabolism and microbial interactions

The degradation of polymeric materials such as polysaccharides, proteins, nucleic acids and lipids into CO_2 and methane involves a complex microbial community in which the metabolic capabilities are combined [42]. The mutual dependence between interacting species can be so extreme that neither species can function without the activity of its partner, and, together, the partners perform functions that neither species could perform alone.

Methanogenesis is performed by anaerobic consortia that degrade biological compounds, including lipids, carbohydrates, and proteins [43], as shown in Table 1. Most of the knowledge on methanogens comes from investigations in both natural and artificial methanogenic environments, such as freshwater sediments and anaerobic reactors. However, these consortia are also functional in the gastrointestinal tracts of humans and animals, and possibly in the oral cavity. This evidence came from studies showing that methane produced in the digestive tract is absorbed into the bloodstream and exchanged in the lungs [44]; methane can be detected in

TABLE 1. Production of methane from different classes of substrate [43]

Component	Methanogenic reaction
Lipids	$C_{50}H_{90}O_6 + 24.5H_2O \rightarrow 34.75CH_4 + 15.25CO_2$
Carbohydrates	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$
Proteins	$C_{16}H_{24}O_5N_4+14.5H_2O \rightarrow 8.25CH_4+3.75CO_2+4NH_4^+ +4HCO_3^-$

the breath of approximately one-quarter of the adult human population [45].

Fermentative bacteria hydrolyse the polymeric substrates and ferment the hydrolysis products into fatty acids, CO_2, and H_2 (Fig. 1). Methanogens obtain energy by reducing CO_2 and other simple substrates to methane, with H_2 as the primary electron donor [46]. Therefore, they depend on other microorganisms that degrade more complex organic compounds for substrate supply.

In anoxic environments, full degradation of low molecular weight fatty acids such as butyrate, propionate and acetate occurs only when the H_2 partial pressure is kept very low [47]. In microbial communities, H_2-producing bacteria and H_2-utilizing methanogens sense environmental conditions, influencing each other's metabolism. At high concentrations of hydrogen, the metabolism of the hydrogen producer is inhibited, whereas that of the methanogen is stimulated, and vice versa [46]. It is not only the consumption of H_2 and CO_2 by methanogens, but also the activity of other hydrogenotropic groups, as sulphate reducers and acetogenic bacteria, that provides the conditions for the further degradation of the substrate by the microorganisms involved in the first steps of the food chain. The cooperation between H_2-utilizing microorganisms such as methanogens and H_2 producers leads to substrate degradation while keeping the H_2 concentration low [48,49]. Thus, the removal of end-products by methanogens favours the growth of fermenting bacteria [50], whereas high partial pressures of H_2 would reduce the fermentation efficiency [51], as summarized in Fig. 1.

The balance among members of a complex microbial community may also be affected by other existing conditions, such as nutrient availability and pH and O_2 levels. For instance, when electron acceptors other than CO_2 are present, such as O_2, NO_3^-, Fe^{2+}, and SO_4^{2-}, methanogens are outcompeted by bacteria such as sulphate-reducing bacteria (SRB), denitrifying bacteria, and iron-reducing bacteria. This phenomenon probably occurs because these compounds are better electron acceptors, and their reductions are thermodynamically more favourable than CO_2 reduction to methane [42]. However, it seems that methane-producing organisms

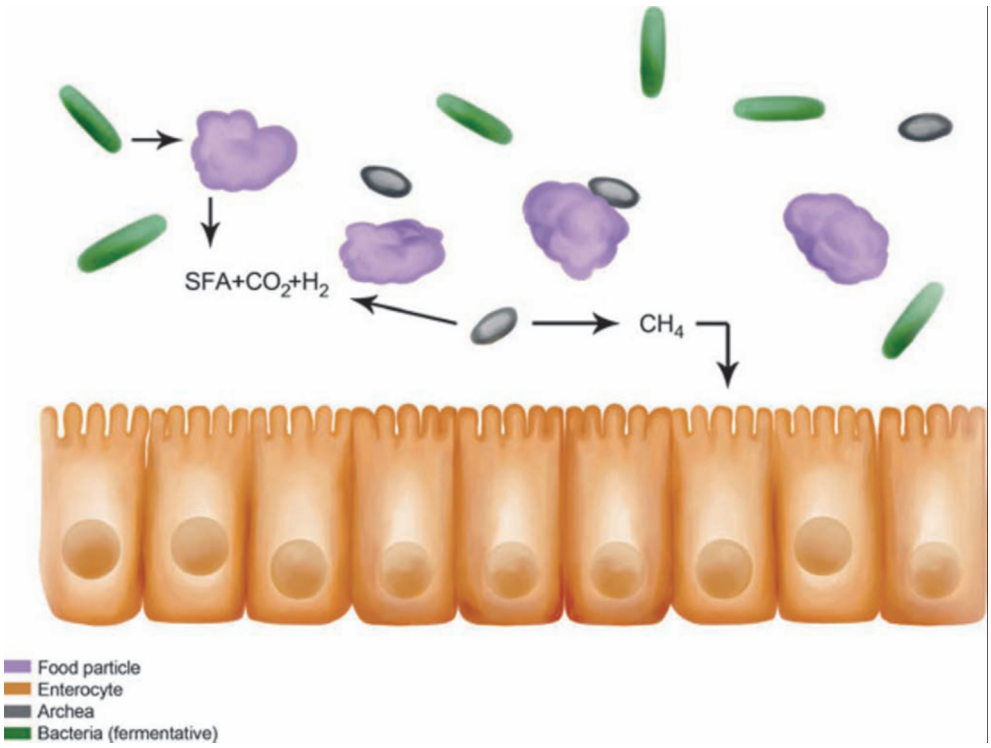

FIG. 1. In the intestine, food particles are degraded by fermentative bacteria, resulting in the production of byproducts such as short-chain fatty acids (SFA), CO_2, and H_2. Then, methanogens obtain energy by reducing CO_2 to methane, with H_2 as the primary electron donor.

can coexist with SRB at comparable levels of abundance in the human colon [52] and mouth [33].

Methanogens could also outcompete with homoacetogens, as the latter can reduce CO_2 for energy production. However, homocetogens do not compete well with methanogens in many habitats, such as the colon [42], as acetogenesis with H_2 is thermodynamically less favourable than methanogenesis. On the other hand, homoacetogens may have advantages over methanogens, owing to their metabolic versatility, such as lower sensitivity to O_2.

Archaea in humans

As methanogenic archaea are involved in a metabolic cascade, they are usually part of a complex biosystem. Archaea, bacteria and eukaryotic cells coexist in a symbiotic relationship in human mucosal surfaces, such as the intestine [53] and the oral cavity [33]. Archaea have been found in the vaginas of some women with bacterial vaginosis [26], but not in the amniotic fluid of women in preterm labour [54,55].

Despite the fact that the intestine is the largest immune organ of the body, containing 80% of the human antibody-producing cells [56], it harbours at least 500–1000 different bacterial species [35].

After birth, the oxygenated neonatal gut is first colonized by facultative bacteria. In about 1 week, the oxygen is consumed, favouring the establishment of anaerobic bacteria [57]. The methanogenic archaeon *M. smithii* was first identified in 1982 from a human faecal culture [24]. It may be transiently present in the infant gut [14,58], possibly originating from the mother's vaginal microbiota [26]. However, it may become part of the normal resident microbiota of the intestines in children older than 27 months [59]. This colonization pattern seems to have no association with dietary habits, but rather results from environmental contamination [9].

Methanogenic archaea can be detected in approximately 25–40% of children and 42–82% of adults [59,60], but its prevalence may be higher. Indeed, a more recent study reported that 95.5% of the subjects were colonized by *M. smithii*, leading to the suggestion that this organism could be used as an indicator of faecal contamination in water [14].

Methanogenic archaea can constitute up to 10% of all anaerobes in the colon of healthy adults [35], and may play a role as a symbiont in the intestines by preventing the accumulation of bacterial end-products [61]. On the other hand, a role in pathogenesis for methanogenic archaea has been suggested. An increase in methane breath has been associated with malignant or premalignant colonic pathology [29], inflammatory bowel disease [13,62], and constipation [45]. Other studies associated an increased proportion of archaea with inflammatory bowel disease (or Crohn's disease), irritable bowel syndrome, colorectal cancer, and diverticulosis [63,64]. The mechanism underlying these pathologies is still unclear; disease does not seem to be associated with a particular pathogen, but rather with a microbial shift towards an anaerobic consortium, consisting of saccharolytic and proteolytic strictly anaerobic bacteria, including *Clostridium*, *Eubacterium*, the *Bacteroides*/*Prevotella* cluster [65,66], the terminal-degrading methanogens [13,62], and SRB [65], with a concomitant reduction in probiotic bacteria. In addition, methanoarchaea in the intestines may enhance fibre digestion and thus calorie intake, as shown in a humanized gnotobiotic mouse model [67]. Thus, colonization by methanoarchaea may be helpful in malnourished subjects, but harmful in obesity.

The distribution of the oral microbiota is affected by several environmental conditions, including redox potential and nutrient availability. Early dental biofilm colonizers are mainly facultative anaerobic saccharolytic bacteria, such as *Actinomyces* and *Streptococcus*. These species consume O_2 and open the way to the dominance of strictly anaerobic secondary fermenters, such as *Fusobacterium* and *Prevotella* species. This microbial succession affects the habitat, with a further drop in the redox potential and the production of gingival fluid, which contains complex host molecules that can be exploited as primary nutrient sources by proteolytic anaerobes [68]. Then, strictly anaerobic, proteolytic bacteria as *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* outcompete the early colonizers and induce gingival tissue destruction [69].

Our research group has been investigating a possible interaction between archaea and bacteria in periodontal disease. As expected, in the oral cavity, methanogens were mainly detected in the highly anoxic environment of subgingival biofilm and in infected root canals [36,70]. Under these conditions, methane-producing archaea would possibly remove end-products, lowering the concentration of H_2 and possibly favouring fermenters. The presence in the subgingival dental plaque of SRB, which use sulphate as electron acceptor and thus compete with methanogens in most environments, has been previously associated with increased proportions of the periodontopathogens *P. gingivalis*, *T. forsythia*, and *Treponema denticola* [71].

Both SRB and methanogenic archaea are terminal degraders in complex anaerobic systems. Methanogenic archaea are also present in higher counts and proportions in diseased than in healthy sites of aggressive periodontitis subjects [72], and they coexist with a microbiota formed by saccharolytic bacteria such as *Capnocytophaga* sp., *Eubacterium nodatum*, and *Streptococcus constellatus*, and proteo-

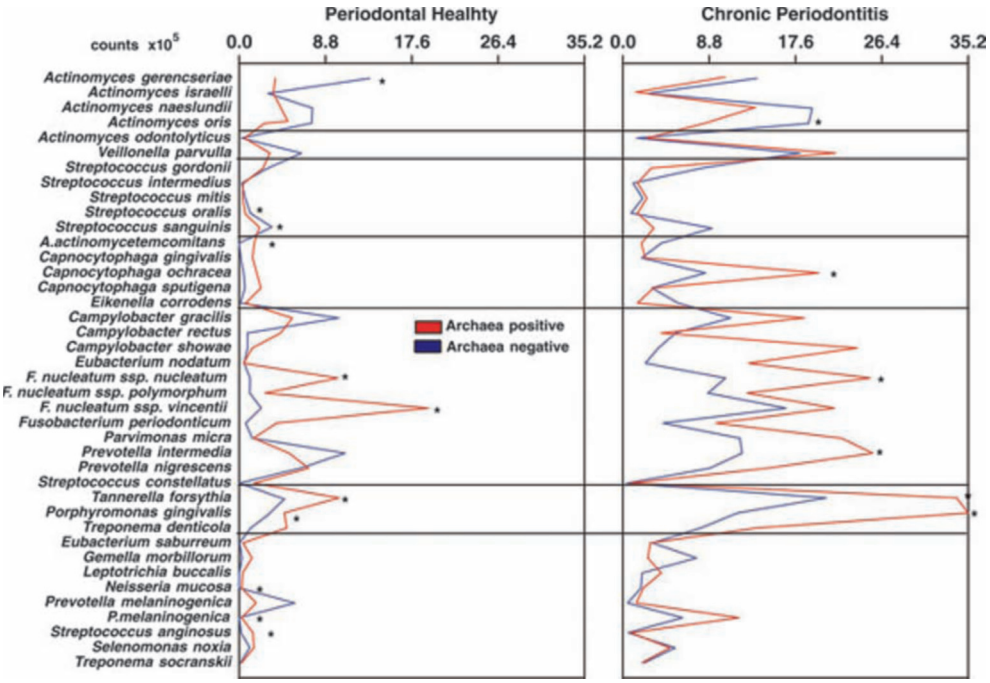

FIG. 2. Mean counts ($\times 10^5$) of 40 bacterial species in subgingival plaque samples taken from 15 periodontally healthy (PH; left panel) and 15 chronic periodontitis (ChP; right panel) subjects. The levels and proportions of archaea were analysed by quantitative PCR and the microbial profile was analysed by checkerboard DNA–DNA hybridization in six and three samples/subject in the ChP and PH groups, respectively. The significance of differences between sites colonized by archaea in each clinical group was assessed with the Wilcoxon test ($^*p < 0.05$).

lytic bacteria such as *P. gingivalis* and *T. forsythia* [73]. Recent data (unpublished) demonstrated a positive correlation between the levels of archaea and *P. gingivalis* (r = 0.75, p 0.001), as well as *T. forsythia* (r = 0.65, p 0.01), in the subgingival biofilm of chronic periodontitis subjects. These data are shown in Fig. 2. On the other hand, periodontally healthy sites colonized by archaea had lower levels of beneficial species of the genera *Actinomyces* and *Streptococcus*, whereas some putative periodontal pathogen species were found in significantly higher levels and proportions than in sites not colonized by these microorganisms. A positive association between methanogens and *Synergites* spp., an anaerobic group of bacteria, which are mostly not yet cultivable, was also reported [36,74]. Thus, it seems that methanogenic archaea and SRB coexist in the oral cavity with periodontal pathogens, and may play a role as terminal degraders of host components, favouring a continuous catabolic cascade.

Inflammatory diseases in the gut [75] and in the oral cavity [69] are induced by unbalanced microbial communities, with a depletion in commensal bacteria and an increase in the anaerobic consortia, which include true and opportunistic bacterial pathogens, as well as archaea. By using up hydrogen, methanogens benefit from bacterial byproducts, but they provide conditions for the growth of anaerobic fermenting bacteria, including the more virulent ones [21,52]. Even though the use of antibiotics targeting bacteria is successful in limiting the damage promoted by microbial consortia in the gut and oral cavity [76,77], antibacterial agents probably disrupt the microbial food chain, limiting H_2, and lead to an increase in the redox potential, thus preventing the survival of anaerobic terminal degraders, the methanoarchaea. Furthermore, methanogens have the ability to transform heavy metals, or metalloids, into volatile methylated derivatives, which are more toxic than the original compounds, increasing tissue damage [21,78].

Members of the domain *Archaea* form part of the resident microbiota in humans, and may be involved in the microbial shifts seen in diseased sites, but the clinical relevance of archaea remains unknown.

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Transparency Declaration

The authors declare no conflicts of interest.

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